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Effects of Metformin-Rosiglitazone on Glucose, Coagulation and Fibrinolysis among Diabetics

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Abstract

Diabetes mellitus (DM) is a group of metabolic diseases in which there is chronic hyperglycaemia. Diabetes is due to either poor islet cells functions or defects in glucose receptors. Symptoms of DM include frequent urination, increased thirst, and increased hunger. If left untreated or poorly treated, diabetes can cause many complications. There is a high risk of atherosclerosis and coronary artery disease in people with diabetes. This phenomenon is largely explained by a deregulation of factors involved in coagulation and platelet activation. The effects of improved glycemic control on PAI-1 levels in type 2 diabetes have been demonstrated for different oral antidiabetic therapies, such as Metformin alone or in combination with Rosiglitazone. Metformin decreases hyperglycemia primarily by suppressing glucose production by the liver. Metformin and Rosiglitazone have a favorable effect on the coagulation system in diabetes. Therefore, routine examinations of Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT) and other coagulation parameters are important to assess coagulation impairment in diabetes in order to prevent thromboembolic cardiovascular disease. This paper highlights the efficacy of metformin-rosiglitazone combination therapy for management of type 2 diabetes and its effect on coagulation and fibrinolysis.

Keywords: Fibrinolysis, coagulation, diabetes, metformin, treatment.

Introduction

Diabetes

Diabetes mellitus is a group of metabolic diseases in which there is chronic hyperglycaemia (Morrish *et al.*, 2001). Symptoms of high blood glucose include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications (Roglic *et al.*, 2005). Acute complications include diabetic ketoacidosis and non-ketotic hyperosmolar coma. Serious long-term complications include cardiovascular disease, stroke, chronic kidney failure, foot ulcers, and damage to the eyes. Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced (David, 2011).

Type 2 Diabetes

Type 2 diabetes begins with insulin resistance, a condition in which cells fail to respond to insulin properly (Roglic *et al.*, 2005). As the disease progresses a lack of insulin may also develop (Tripathy *et al.*, 2012).

Insulin is the principal hormone for glucose metabolism that regulates the uptake of glucose from the blood into most cells of the body, especially liver, muscle, and adipose tissue. Therefore, deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes. Insulin is released into the blood by beta cells (β -cells), found in the islets of Langerhans in the pancreas, in response



to rising levels of blood glucose, typically after eating. Lower glucose levels result in decreased insulin release from the beta cells and in the breakdown of glycogen to glucose. This process is mainly controlled by the hormone glucagon, which acts in the opposite manner to insulin (Kim, 2012). If the amount of insulin available is insufficient, if cells respond poorly to the effects of insulin (insulin insensitivity or insulin resistance), or if the insulin itself is defective, then glucose will not be absorbed properly by the body cells that require it, and it will not be stored appropriately in the liver and muscles. The net effect is persistently high levels of blood glucose, poor protein synthesis, and other metabolic derangements, such as acidosis (David, 2011).

Coagulation

Coagulation (also known as clotting) is the process by which blood changes from a liquid to a gel, forming a clot. It potentially results in haemostasis, the cessation of blood loss from a damaged vessel, followed by repair. The mechanism of coagulation involves activation, adhesion, and aggregation of platelets along with deposition and maturation of fibrin. Disorders of coagulation are disease states which can result in bleeding (haemorrhage or bruising) or obstructive clotting (thrombosis) (David *et al.*, 2009). Both a cellular (platelet) and a protein (coagulation factor) component are involved.

Coagulation begins almost instantly after an injury to the blood vessel has damaged the

endothelium lining the vessel. Exposure of blood to the space under the endothelium initiates two processes: changes in platelets, and the exposure of sub endothelial tissue factor to plasma Factor VII, which ultimately leads to fibrin formation. Platelets immediately form a plug at the site of injury; this is called primary haemostasis. Secondary haemostasis occurs simultaneously: Additional coagulation factors or clotting factors beyond Factor VII respond in a complex cascade to form fibrin strands, which strengthen the platelet plug (Furie, 2005).

Role of Platelets

When the endothelium is damaged, the normally isolated, underlying collagen is exposed to circulating platelets, which bind directly to collagen with collagen-specific glycoprotein Ia/IIa surface receptors. This adhesion is strengthened further by von Willebrand factor (vWF), which is released from the endothelium and from platelets; vWF forms additional links between the platelets glycoprotein Ib/IX/V and the collagen fibrils. This localization of platelets to the extracellular matrix promotes collagen interaction with platelet glycoprotein VI. Binding of collagen to glycoprotein VI triggers a signaling cascade that results in activation of platelet integrins. Activated integrins mediate tight binding of platelets to the extracellular matrix. This process adheres platelets to the site of injury (Nigel *et al.*, 2009). Activated platelets will release the contents of stored granules into the blood plasma (Fig. 1).

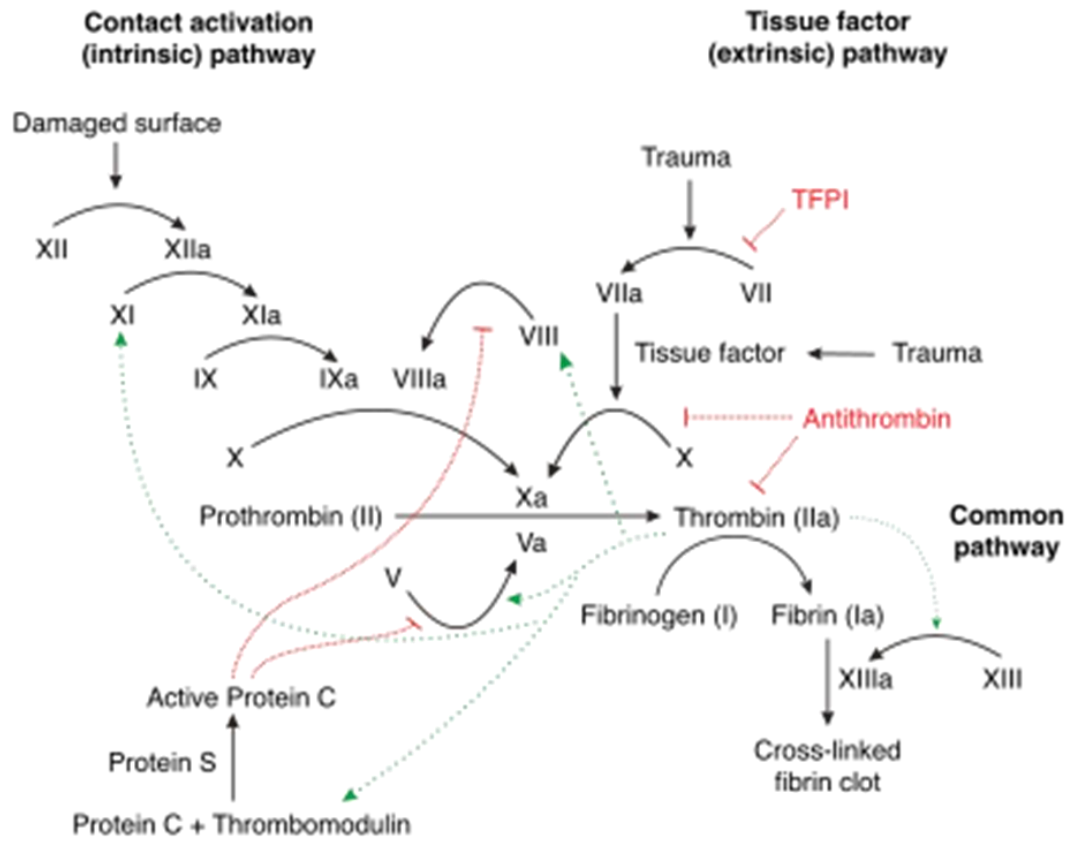


Figure 1. The classical blood coagulation pathway (Pallister and Watson, 2010).

The coagulation cascade

The coagulation cascade of secondary haemostasis has two initial pathways which lead to fibrin formation. These are the contact activation pathway (also known as the intrinsic pathway), and the tissue factor pathway (also known as the extrinsic pathway) which both lead to the same fundamental reactions that produce fibrin (Fig.1). The pathways are a series of reactions, in which a zymogen (inactive enzyme precursor) of a serine protease and its glycoprotein co-factor are activated to become active components that then catalyze the next reaction in the cascade, ultimately resulting in cross-linked fibrin (Pallister and Watson,2010).

Complications in Diabetes

Diabetes is associated with an increased risk of atherosclerosis, and coronary artery disease is a major cause of death in patients with diabetes. Thrombosis is the cause of death in 80% of patients with diabetes. Of these deaths, 75% are

due to cardiovascular complications, while the remaining 25% are due to cerebrovascular events and peripheral vascular complications (Carr, 2001). It is well known that diabetes changes the thrombo-haemorrhagic balance that exists in healthy flowing blood. This change predisposes the diabetic patient to various thromboembolic conditions, leading to increased mortality and morbidity (Biondi-Zoccai *et al.*, 2003). In the context of insulin resistance, the result is an exaggeration of the mitogenic actions of insulin, leading to vascular smooth muscle proliferation and an elevated level of plasminogen activator inhibitor-1 (PAI-1) (Reusch,2002), the major inhibitor of endogenous thrombolysis, thereby promoting thrombosis. Other factors that may be involved in the thrombophilic status associated with diabetes include increased plasma levels of coagulation factors XII, XI and VIII, platelet hypersensitivity to collagen and an increased circulating pool of tissue factor (Sambola *et al.*, 2003) in diabetes plus metabolic syndrome.



Fibrinolysis

Fibrinolysis is a process that prevents blood clots from growing and becoming problematic (Dugdale *et al.*, 2011). This process has two types: primary fibrinolysis and secondary fibrinolysis. The primary type is a normal body process, whereas secondary fibrinolysis is the breakdown of clots due to a medicine, a medical disorder, or some other cause (Dugdale *et al.*, 2011). In fibrinolysis, a fibrin clot, the product of coagulation, is broken down. Its main enzyme plasmin cuts the fibrin mesh at various places, leading to the production of circulating fragments that are cleared by other proteases or by the kidney and liver.

Plasmin is produced in an inactive form, plasminogen, in the liver. Although plasminogen cannot cleave fibrin, it still has an affinity for it, and is incorporated into the clot when it is formed. Tissue plasminogen activator (t-PA) (Cotran *et al.*, 2005) and urokinase are the agents that convert plasminogen to the active plasmin, thus allowing fibrinolysis to occur. t-PA is released into the blood very slowly by the damaged endothelium of the blood vessels, such that, after several days (when the bleeding has stopped), the clot is broken down. This occurs because plasminogen became entrapped within the clot when it formed; as it is slowly activated, it breaks down the fibrin mesh. t-PA and urokinase are themselves inhibited by plasminogen activator inhibitor-1 and plasminogen activator inhibitor-2 (PAI-1 and PAI-2). In contrast, plasmin further stimulates plasmin generation by producing more active forms of both tissue plasminogen activator (t-PA) and urokinase (Cotran *et al.*, 2005). Alpha 2-antiplasmin and alpha 2-macroglobulin inactivate plasmin. Plasmin activity is also reduced by thrombin-activatable fibrinolysis inhibitor (TAFI), which modifies fibrin to make it more resistant to the tPA-mediated plasminogen (Cesarman-Maus and Hajjar, 2005).

Metformin (Biguanide)

Metformin is the only drug belonging to the biguanide class currently available in most parts of the world (Fisman and Tenenbaum, 2008).

Metformin as an insulin sensitizer, inhibits gluconeogenesis, decreases glucose absorption from gastrointestinal system (GIS) and increases the peripheral glucose uptake by the tissues (mainly skeletal muscles) in the presence of insulin (Misbin *et al.*, 1998). Metformin promotes glucose control in two ways: (a) by decreasing hepatic glucose release through an inhibition of gluconeogenesis, and (b) by increasing the sensitivity of peripheral tissue to insulin (Stumvoll *et al.*, 1995).

Mechanism of Action

Metformin decreases hyperglycemia primarily by suppressing glucose production by the liver (hepatic gluconeogenesis) (Kirpichnikov *et al.*, 2002). The "average" person with type 2 diabetes has three times the normal rate of gluconeogenesis; metformin treatment reduces this by over one-third (Hundal *et al.*, 2000). The molecular mechanism of metformin is incompletely understood: inhibition of the mitochondrial respiratory chain (complex I), activation of AMP-activated protein kinase (AMPK), inhibition of glucagon-induced elevation of cyclic adenosine monophosphate (cAMP), and consequent activation of protein kinase A (PKA), inhibition of mitochondrial glycerophosphate dehydrogenase, and an effect on gut microbiota have been proposed as potential mechanisms (Burcelin, 2013; Madiraju *et al.*, 2013; Rena *et al.*, 2013).

Rosiglitazone (Thiazolidinedione)

Thiazolidinediones (often referred to colloquially as 'glitazones') are oral antidiabetic agents widely used to treat patients with type 2 diabetes mellitus (T2DM) that act primarily as insulin sensitizers, improving glucose control and decreasing insulin resistance. Rosiglitazone and pioglitazone are two compounds in this class of insulin sensitizing drugs presently approved for use in many countries as monotherapy or in combination with metformin, sulfonylureas or insulin. It is well established that they enhance peripheral glucose uptake and improve insulin sensitivity in patients with type 2 diabetes (Martines *et al.*, 2002) and prevent progression to T2DM in patients with impaired

glucose tolerance and insulin resistance (Durbin, 2004).

Mode of Action of Thiazolidinediones

Thiazolidinediones act on adipose tissue to decrease plasma free fatty acids (FFAs) and secretion of tumour necrosis factor- α (TNF- α) and resistin, often increased with obesity and diabetes, therefore improving insulin action in

muscle, adipose tissue and liver. They are believed to increase insulin sensitivity by activating highly selective and potent nuclear receptors, the peroxisome proliferator-activated receptor-gamma (PPAR γ), thereby directly regulating gene expression as a result of which peripheral glucose uptake is increased and hepatic glucose production is diminished (Kersten *et al.*, 2000) (Fig. 2).

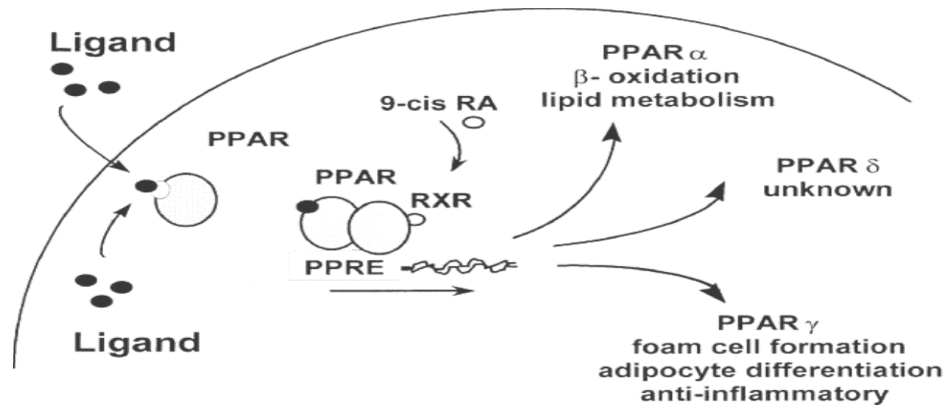


Figure 2: Activation of the PPAR receptors leading to the transcription of the responsive gene (David, 2000). PPAR=peroxisome proliferator-activated receptor, RA=retinoic acid, PPRE= peroxisome proliferators response element, RXR= retinoid X receptor.

Diabetes and Thrombosis Risk

The underlying mechanism for increased thrombosis risk in diabetes are complex and involve multiple pathways. Subjects with diabetes have premature atherosclerosis and more extensive vascular disease, predisposing them to plaque rupture and thrombus formation (Stratmann and Tschoepe, 2009). In addition, these individuals have increased thrombotic tendency due to platelets hyper-reactivity and increased activation of prothrombotic coagulation factors coupled with decreased fibrinolysis (Grant, 2007).

Thrombosis and Coagulation

Individuals affected by diabetes display an increased risk of coronary events and cardiovascular mortality when compared with non-diabetic subjects (Roffi *et al.*, 2011; Radke and Schunkert, 2010). This phenomenon is

largely explained by a deregulation of factors involved in coagulation and platelet activation (Vazzana *et al.*, 2012). Both insulin resistance and hyperglycemia participate to the pathogenesis of this prothrombotic state (Beckman *et al.*, 2002). Insulin resistance increases PAI-1 and fibrinogen and reduces tissue plasminogen activator levels. The largest increase in PAI-1 has been reported in diabetic patients with poor glycaemic control and treatment with glucose-lowering agents like metformin, comparably decreased PAI-1 (Lemkes *et al.*, 2010). Hyperinsulinemia induces TF expression in monocytes of patients with type 2 diabetes leading to increased TF procoagulant activity and thrombin generation (Boden and Rao, 2007). These events are enhanced by hyperglycemia (Boden and Rao, 2007).

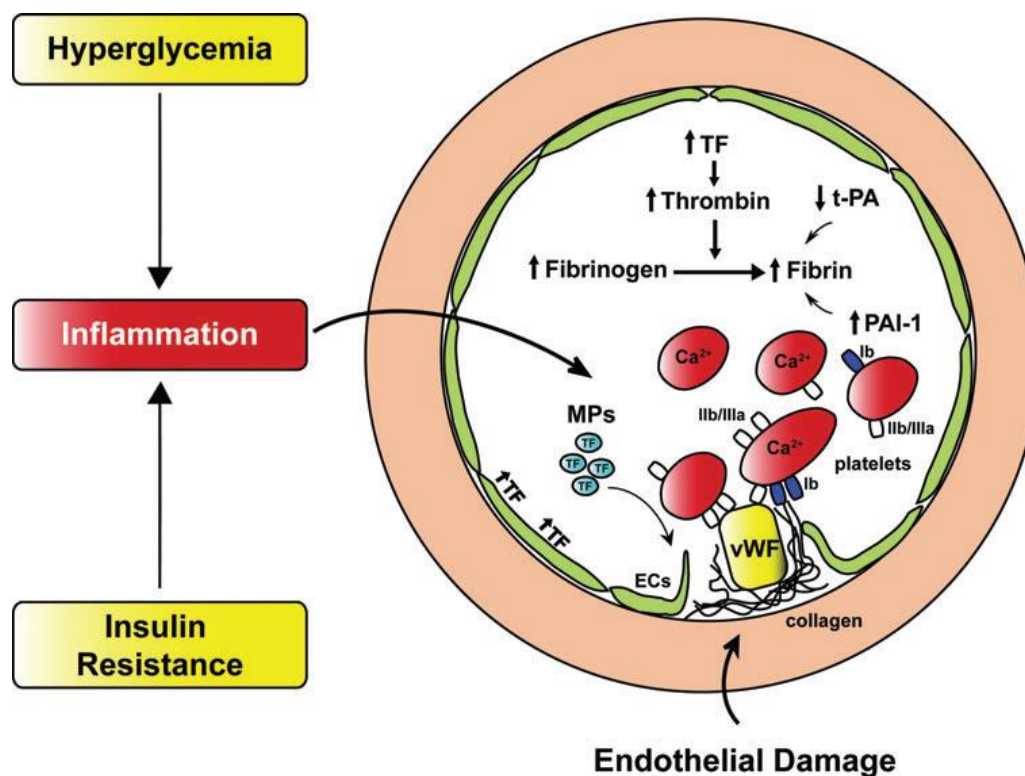


Figure 3. Coagulation and platelet reactivity in diabetes. (Francesco *et al.*, 2013).

TF, tissue factor; t-PA, tissue plasminogen activator; PAI-1 plasminogen activator inhibitor-1; vWF, von Willebrand factor; ECs, endothelial cells; MP, microparticles; Ca^{2+} , calcium.

Clot Formation and Lysis

Fibrin clot formation, leads to vessel occlusion, represents the final step in the atherothrombotic process and involves multiple interactions between coagulation factors and platelets (Ajjan and Grant, 2005). Rupture or fissuring in atherothrombotic plaques exposes a prothrombotic core that comes into contacts with platelets and coagulation factors. Initially, platelets adhere to the site of break and become partially activated. Factor VII (FVII) binds to exposed tissue factor (TF) and form a complex, which subsequently activates FIX and FX, resulting in FV activation. Activated FX and FV cleave prothrombin to generate limited amounts of thrombin, enough to maintain platelets activation but insufficient to sustain thrombus formation. Platelets stimulation by thrombin and exposed collagen results in full activation and degranulation, followed by FV release and activation by both FXa and thrombin. Thrombin also activates FVIII and this can form an active complex with FIXa, further enhancing the coagulation process. Subsequently, enough thrombin is generated to convert soluble

fibrinogen into a network of fibrin fibres. More stabilization of the clot is achieved by thrombin activated factor XIII which crosslinks adjacent fibrins fibres and incorporates other proteins such as $\alpha 2$ -antiplasmin and fibronectin into the network, thereby increasing clot stability and resistance to lysis (Ariens *et al.*, 2002).

A defense mechanism against thrombosis occurs via natural anti-coagulants, such as protein C, which inhibits factor V and VIII, and tissue factor pathway inhibitors that blocks TF-FVII initiation of coagulation (Ajjan and Ariens, 2009). Tissue plasminogen activator (t-PA), produced by endothelial cells, mediates the conversion of plasminogen to plasmin, which starts the fibrinolytic process. Plasmin cleaves fibrin fibres in different locations, resulting in the formation of fibrin degradation products (FDPs) of various sizes. An important protein that inhibits fibrinolysis is plasminogen activator inhibitor-1 (PAI-1), which blocks the conversion of plasminogen into active plasmin (Figure 4).

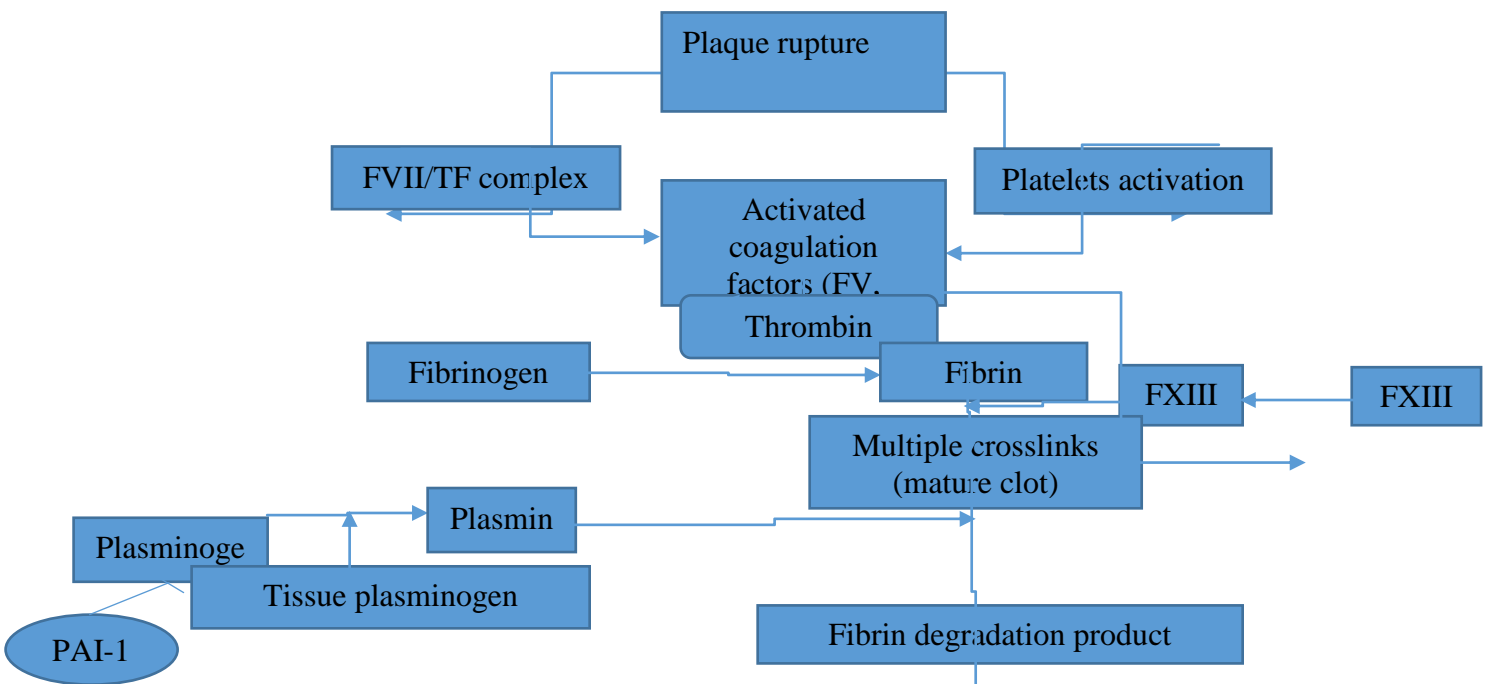


Figure 4. Diagram summarizing the coagulation cascade and fibrin clot formation.TF, tissue factor; PAI-1, plasminogen activator inhibitor-1. (Alzahrani *et al.*, 2010).
Markers of Coagulation and Fibrinolysis

Both parameters of increased coagulability as well as a fibrinolytic impairment have been found in type 2 diabetes. Although, there are many different markers in the circulation to measure these abnormalities. Platelet-dependent thrombin generation, for instance, was measured in patients with poor glycemic control, good glycaemic control and healthy controls. In vitro induced thrombin generation was found to be increased in platelet-rich plasma from diabetes patients compared to healthy controls and a significant elevation of thrombin levels was also demonstrated in plasma from poorly controlled type 2 diabetes when compared to well controlled patients (Aoki *et al.*, 1996). In a placebo-controlled trial using glitazone (rosiglitazone) combined with diet modification a significant association was shown between improved glycaemic control and blood thrombogenicity as reflected by a reduction in *ex-vivo* thrombus formation in a Badimon perfusion chamber. Improved glycemic control was the only significant predictor of a decrease in blood

thrombogenicity irrespective of treatment allocation (Osende *et al.*, 2001).

Changes in Coagulation Factors in Type 2 Diabetes and Potential Mechanism

In a group of poorly controlled type 2 diabetic patients (HbA1c 10%) extraordinarily high concentrations of plasminogen activator inhibitor-1 (PAI-1), indicating hypofibrinolysis, were detected leading the authors to conclude that profound hyperglycemia is accompanied by profound increases in PAI-1. Subsequent treatment of hyperglycemia with metformin as monotherapy comparably decreased PAI-1, which argues for an effect of glucose lowering rather than a specific medication effect (Cefalu *et al.*, 2002). This state of hypofibrinolysis was recently confirmed in a case-control study; patients with type 2 diabetes were found to have a prolonged clot lysis time as well as elevated levels of PAI-1 and von Willebrand factor (vWF) (Verkleij *et al.*, 2009). The impairment in the fibrinolytic system in type 2 diabetes of interest since these impairments are independent primary risk factors for myocardial infarction



(Meltzer *et al.*, 2009; Thogersen *et al.*, 1998). Coagulation activation markers, as indicated by many studies, such as antithrombin-thrombin complexes (TATc) and prothrombin activation fragment 1+2(F1+2) are elevated in diabetes (Yamagashi *et al.*, 2007; Yokoyama *et al.*, 1996). Plasma levels of many clotting factors including fibrinogen, factor VIII, factor XI, factor XII, kallikrein, and vWF), are elevated in

diabetes. Conversely, the level of anticoagulant protein C is decreased (Ceriello *et al.*, 1990). The fibrinolytic system, the primary means of removing clots, is relatively sluggish in diabetes due to abnormal clot structure that is more resistant to degradation and an increase in PAI-1 which inhibits tissue plasminogen activator (t-PA) (Erem *et al.*, 2005; Aso *et al.*, 2002).

Table 1: Coagulation factors, level and activity in type 2 diabetes (Alzahrani and Ajjan, 2010)

Coagulation factors	Production	Function	Level/activity	Possible mechanism
TF	Stimulated EC, VSMC	Initiate clot formation	↑	↑Insulin, ↑glucose, ↑AGE, ↑ROS (Breitenstein <i>et al.</i> ,2010; Annex <i>et al.</i> , 1995; Boden <i>et al.</i> , 2007)
FVII	Liver	Initiate clot formation	↑	↑glucose, ↑triglyceride (Karatela and Sainani,2009; Heinrich <i>et al.</i> , 1994)
(Pro)thrombin	Liver	Fibrinogen→fibrin	↑	↑glucose (Boden <i>et al.</i> , 2007; Ceriello <i>et al.</i> , 2009; Wolberg and Campbell, 2008)
Fibrinogen	Liver	fibrin network	↑	↑liver synthesis, IR (Tessari <i>et al.</i> , 2006;Barazzoni <i>et al.</i> , 2003; Klein <i>et al.</i> , 2003)
t-PA	EC	Clot lysis (Plasminogen→plasmin)	↓	EC dysfunction (Juhan-Vague <i>et al.</i> , 1996; Lowe <i>et al.</i> ,2004; Sahli <i>et al.</i> , 2009)
PAI-1	EC,VSMC,adipocyte,liver	Inhibit clot lysis	↑	↑glucose, IR (Seljeflot <i>et al.</i> , 2006; Stegenga <i>et al.</i> , 2008).

EC, endothelial cells; VSMC, very smooth muscle cells; AGEs, advanced glycation end products; ROS, reactive oxygen species; FVII, factor VII; IR, insulin resistance, t-PA, tissue plasminogen activator, PAI-1, plasminogen activator inhibitor-1; ↑ increase; ↓decrease; →convert.

Medical Laboratory Assessment of Coagulation Impairment in Type 2 Diabetes

Coagulation abnormalities with decreased level of antithrombin III, protein C and protein-S has been reported in Type 2 diabetes with elevated clotting factors (Madan *et al.*, 2010). The following laboratory tests could be used to assess degree of coagulation impairment in type 2 diabetes:

1. Prothrombin time (PT)
2. Activated partial thromboplastin time (APTT)
3. Plasma fibrinogen level (PFL)
4. Euglobulin lysis time (ELT)
5. Clotting factor assay (CLA)
6. Platelet count/Platelet function test (PFT)
7. Fasting lipid profile (FLP)
8. Haemoglobin A_{1c}(HbA_{1c})

Prothrombin time (PT) and APTT are the markers for activation of extrinsic and intrinsic pathway respectively (Dhule and Gawali, 2014; Mwambungu *et al.*, 2013; Alzahrani and Ajjan, 2010). The hypercoagulability state demonstrated by shortened PT and APTT in type 2 diabetes may cause occlusive thrombus within a coronary artery (Ali *et al.*, 2011). Therefore, shortened PT and APTT value are the risk factors for thromboembolic cardiovascular disease in type 2 diabetic patients (Sapkota *et al.*, 2013).

Expected values in coagulation impairment in type 2 diabetes: PT and APTT are shortened (Erem *et al.*, 2005), plasma fibrinogen level is elevated (Asakawa *et al.*, 2000), Euglobulin lysis time is prolonged, platelet count is usually reduced (Linden *et al.*, 2012), glycosylated haemoglobin A_{1c} is usually >10% (Erem *et al.*, 2005). Total cholesterol, triglyceride and LDL-

C are usually elevated with decreased HDL-C (Zhang *et al.*, 2012).

Modulation of Thrombosis Potential in Type 2 Diabetes

In normal circumstances, there is a balanced production/activation of prothrombotic and fibrinolytic factors ensuring appropriate haemostasis, enough to protect from bleeding but adequately suppressed to prevent pathological thrombosis. In diabetes, however, this balance is tipped towards a prothrombotic/hypofibrinolytic phenotype which, coupled with atheromatous vascular changes and platelet hyperactivity, predisposes to increased cardiovascular ischemic events (Alzahrani and Ajjan, 2010).

Effect of Glucose Lowering Therapy (Metformin-Rosiglitazone)

The effects of improved glycemic control on PAI-1 levels in type 2 diabetes have been demonstrated for different oral antidiabetic therapies, such as metformin alone (Fontbonne *et al.*, 1996) or in combination with rosiglitazone (Derosa *et al.*, 2006) and glimepiride in combination with pioglitazone or rosiglitazone (Derosa *et al.*, 2005). Metformin, which already had proven beneficial cardiovascular effects in the United Kingdom Prospective Diabetes Study trial (UKPDS), also reduced factor VII and fibrinogen levels and shortened clot lysis time (Grant, 2003). When metformin was added to a sulphonylurea derivative result in poorly controlled elderly type 2 diabetes patients, the resulting substantial improvement in glycemic control was accompanied by beneficial changes in markers of platelet function (platelet factor 4 and beta-thromboglobulin), thrombin generation (fibrinopeptide A, prothrombin activation fragment (F1+2), and D-dimer) and fibrinolysis (PAI-1 activity and antigen) (Gregorio *et al.*, 1999).



Table 2: Effects of Antidiabetic Therapies on Coagulation Factors and Clotting Parameters (Alzahrani and Ajjan, 2010).

Agent	Effect
Metformin	↓FVII, ↓PAI-1, ↓FXIII crosslink activity, looser clot structure and enhanced fibrinolysis (Standeven <i>et al.</i> , 2002; Grant, 2003; Alzahrani <i>et al.</i> , 2010)
Rosiglitazone	↓FVII, ↓Fibrinogen, ↓PAI-1, ↑Thrombomodulin (Haffner <i>et al.</i> , 2002; Buckingham, 2005; Chen <i>et al.</i> , 2010; Perriello <i>et al.</i> , 2007)
Insulin	↑PAI-1, ↑Fibrinogen (prothrombotic) (Tessari <i>et al.</i> , 2006; Stegenga <i>et al.</i> , 2008; Alessi and Juhan-Vague, 2008)

↑increase; ↓decrease; FVII factor VII; PAI-1 plasminogen inhibitor-1;

Conclusions

The haemostatic system is altered in diabetes secondary to changes in clotting factor levels and/or activity. There is a general increase in plasma levels of procoagulant factors accompanied by decreased fibrinolytic capacity. The mechanisms for these alterations are complex, with insulin resistance and hyperglycemia being clear culprits. The net result of the above changes is an increased tendency to clot formation, with the fibrin network displaying a compact structure and resistance to fibrinolysis.

A number of measures can reduce the hypercoagulable environment in type 2 diabetes including tight glycaemic control, with avoidance of hypoglycaemia, and improvement in insulin sensitivity. Of the pharmacological agents used, metformin and rosiglitazone have a favorable effect on the coagulation system and both have been shown to reduce cardiovascular events in diabetes. Therefore, routine examinations of PT, APTT and other coagulation parameters are important to assess coagulation impairment in diabetes in order to prevent thromboembolic cardiovascular disease. Further research is needed to disentangle the prothrombotic event in diabetes.

Conflict of interest: There is no conflict of interest associated with this work.

References

- Ajjan, R.A and Ariens, R.A. (2009). Cardiovascular disease and heritability of the prothrombotic state. *Blood Reviews*; **23**: 67-78.
- Alan, D., Michelson. (2006). Platelets. Academic Press. pp. 3–5. ISBN 978-0-12-369367-9.
- Alessi, M.C., Juhan-Vague, I. (2008). Metabolic syndrome, haemostasis and thrombosis. *Thrombosis and Haemostasis*; **99**: 995-1000.
- Ali, S.F., Mohammad, O.W., Elkareem, A.A., Abdul, Aziz, M.K. (2011). Activated partial thromboplastin time in type II Diabetes Mellitus. *Al Neelain Medical Journal*: 63-69.
- Alzahrani, S.H., Hess, K., Baxter, P.D., Standeven, K.S., Price, J.F., Strachan, M., *et al.* (2010). Fibrin clot structure/hypofibrinolysis and relationship with cardiac ischaemia and inflammation markers in older individual with type2 diabetes. *Diabetes*; **70**: 817.
- Alzahrani, SH., Ajjan, RA. (2010). Coagulation and fibrinolysis in diabetes. *Diabetes and Vascular Disease Research*; **7 (4)**: 260-267.
- Annex, B.H., Denning, S.M., Channon, K.M., Sketch, M.H Jr., Stack, R.S., Morrissey, J.H., *et al.* (1995). Differential expression of tissue factor protein in directional atherectomy specimens from patients with

- stable and unstable coronary syndromes. *Circulation*; **91**: 619-622.
- Aoki, I., Shimoyama, K., Aoki, N., Homori, M., Yanagisawa, A., Nakahara, K., *et al.* (1996). Platelet dependent thrombin generation in patients with diabetes mellitus: effects of glycemic control on coagulability in diabetes. *Journal of American College of Cardiologists*; **27** (3): 560-566.
- Ariens, R.A., Lai, T.S., Weisel, J.W., Greenberg, C.S., Grant, P.J. (2002). Role of factor XIII in fibrin clot formation and effects of genetic polymorphisms. *Blood*; **100**: 743-754.
- Asakawa, H., Tokunaga, K., Kawakami, F. (2000). Elevation of fibrinogen and thrombin-antithrombin III complex levels of type 2 diabetes mellitus patients with retinopathy and nephropathy. *Journal of Diabetes Complications*; **14**: 121-126.
- Aso, Y., Matsumoto, S., Fujiwara, Y., Tayama, K., Inukai, T., Takemura, Y. (2002). Impaired fibrinolytic compensation for hypercoagulability in obese patients with type 2 diabetes associated with increased PAI-1. *Metabolism*; **5**: 471.
- Barazzoni, R., Kiwanuka, E., Zanetti, M., Cristini, M., Vettore, M., Tessari, P. (2003). Insulin acutely increases fibrinogen production in individuals with type 2 diabetes but not in individuals without diabetes. *Diabetes*; **52**: 1851-1856.
- Biondi-Zoccai, G.G., Abbate, A., Liuzzo, G., Biasucci, L.M. (2003). Atherothrombosis, inflammation, and diabetes. *Journal of American College of Cardiologists*; **41**: 1071-1077.
- Boden, G., Rao, A.K. (2007). Effects of hyperglycemia and hyperinsulinemia on the tissue factor pathway of blood coagulation. *Current Diabetes Report*; **7**: 223-227.
- Boden, G., Vaidyula, V.R., Homko, C., Cheung, P., Rao, A.K. (2007). Circulating tissue factor procoagulant activity and thrombin generation in patients with type 2 diabetes: effects of insulin and glucose. *Journal of Clinical Endocrine Metabolism*; **92** (11): 4352-4358.
- Breitenstein, A., Tanner, F.C., Luscher, T.F. (2010). Tissue factor and cardiovascular disease: quo vadis? *Circulation Journal*; **74**: 3-12.
- Burcelin, R. (2013). "The antidiabetic gutsy role of metformin uncovered?". *Gut*; **63** (5): 706-707.
- Carr, M.E. (2001). Diabetes mellitus: A hypercoagulable state. *Journal of Diabetes Complications*; **15**: 44-54.
- Cefalu, W.T., Schneider, D.J., Carlson, H.E., Migdal, P., Gan, L.L., Izon, M.P., *et al.* (2002). Effect of combination glipizide GITS/metformin on fibrinolytic and metabolic parameters in poorly controlled type 2 diabetic subjects. *Diabetes Care*; **25** (12): 2123-2128.
- Ceriello, A., Esposito, K., Ihnat, M., Zhang, J., Giugliano, D. (2007). Simultaneous control of hyperglycemia and oxidative stress normalizes enhanced thrombin generation in type 1 diabetes. *Journal of Thrombosis and Haemostasis*; **7**: 1228-1230.
- Ceriello, A., Quatraro, A., Dello, R.P., Marchi, E., Barbanti, M., Milani, M.R., Giugliano, D. (1990). Protein C deficiency in insulin dependent diabetes: a hyperglycemia related phenomenon. *Thrombosis and Haemostasis*; **64**: 104-107.
- Cotran, Ramzi, S., Kumar, Vinay., Fausto, Nelson., Nelso, Fausto., Robbins, Stanley, L., Abbas, Abul, K. (2005). Robbins and Cotran pathologic basis of disease. St. Louis, Mo: Elsevier Saunders:125. ISBN 0-7216-0187-1.
- David, Bishop-Bailey. (2000). Peroxisome proliferator-activated receptors in the cardiovascular system *British Journal of Pharmacology*; **129**: 823-34
- David, G., Gardner, Dolores. (2011). "Chapter 17". Greenspan's basic & clinical endocrinology (9th ed.). New York: McGraw-Hill Medical.
- David, Lillicap., Nigel, Key., Michael, Makris., Denise, O'Shaughnessy. (2009). Practical Hemostasis and Thrombosis. Wiley-Blackwell: 7-16.



- Derosa, G., Dangelo, A., Ragonesi, P.D., Ciccarelli, L., Piccinni, M.N., Pricolo, F., et al. (2006). Effects of rosiglitazone and pioglitazone combined with metformin on the prothrombotic state of patients with type 2 diabetes mellitus and metabolic syndrome. *Journal of International Medical Research*; **34** (5): 545-555.
- Dhule, S., Gawali, S. (2014). Platelet aggregation and clotting time in type 2 diabetic male. *National Journal of Physiology, Pharmacy and Pharmacology*; **4** (2): 96-98.
- Dugdale, David., et al. (2011). "Primary or secondary fibrinolysis", Medline Plus. Retrieved 7 August 2015.
- Durbin, R.J. (2004). Thiazolidinedione therapy in the prevention/delay of type 2 diabetes in patients with impaired glucose tolerance and insulin resistance. *Diabetes and Obesity Metabolism*; **6** (4): 280-285.
- Erem, C., Hacıhasanoglu, A., Çelik, S., Ovalıa, E., Önder, E.H., Ukinça, K., Degere, O., Telatar, M. (2005). Coagulation and Fibrinolysis Parameters in Type 2 Diabetic Patients with and without Diabetic Vascular Complications. *Medicine Principle and Practice*; **14**: 22–30.
- Fisman, E.Z., Tenenbaum, A. (2008). Cardiovascular Diabetology: Clinical, Metabolic and Inflammatory Facets. *Advances in Cardiology*; **45**: 154–170.
- Fontbonne, A., Charles, M.A., Juhan-Vague, I., Bard, J.M., Andre, P., Isnard, F., et al. (1996). The effect of metformin on the metabolic abnormalities associated with upper-body fat distribution. BIGPRO Study Group. *Diabetes Care*; **19** (9): 920-926.
- Furie, B., Furie, B.C. (2005). "Thrombus formation in vivo". *Journal of Clinical Investigation*; **115** (12): 3355–3362.
- Gregorio, F., Ambrosi, F., Manfrini, S., Velussi, M., Carle, F., Testa, R., et al. (1999). Poorly controlled elderly Type 2 diabetic patients: the effects of increasing sulphonylurea dosages or adding metformin. *Diabetes Medicine*; **16** (12): 1016-1024.
- Heinrich, J., Belleisen, L., Schulte, H., Assmann, G., van de Loo., J. (1994). Fibrinogen and factor VII in the prediction of coronary risk. Results from the PROCAM study in healthy men. *Arteriosclerosis and Thrombosis*; **14**: 54-59.
- Hundal, R., Krssak, M., Dufour, S., Laurent, D., Lebon, V., Chandramouli, V., Inzucchi, S., Schumann, W., Petersen, K., Landau, B., Shulman, G. (2000). Mechanism by which metformin reduces glucose production in type 2 diabetes. *Diabetes*; **49** (12): 2063–9.
- Juhan-Vague, I., Pyke, S.D., Alessi, M.C., Jespersen, J., Haverkate, F., Thompson, S.G. (1996). Fibrinolytic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. ECAT Study Group. European Concerted Action on Thrombosis and Disabilities. *Circulation*; **94**: 2057-2063.
- Kersten, S., Desvergne, B., Wahli, W. (2000). Roles of PPARs in health and disease. *Nature*; **405**: 421-424.
- Kim, E.B. (2012). Ganong's review of medical physiology. (24th ed.). New York: McGraw-Hill Medical.
- Kirpichnikov, D., McFarlane, S.I., Sowers, J.R. (2002). Metformin: an update. *Annals of International Medicine*; **137** (1): 25–33.
- Klein, R.L., Hunter, S.J., Jenkins, A.J., Zheng, D., Semler, A.J., Clore, J., et al. (2003). Fibrinogen is a marker for nephropathy and vascular disease in type 1 diabetes: study of plasma fibrinogen and fibrinogen gene polymorphism in DCCT/EDIC cohort. *Diabetes Care*; **26**: 1439-1448.
- Liu, S., Willett, W.C. (2002). Dietary glycemic load and atherothrombotic risk. *Current Atherosclerosis Report*; **4**: 454–461.
- Lowe, G.D., Danesh, J., Lewington, S., Walker, L., Thomson, A., et al. (2004). Tissue plasminogen activator antigen and coronary heart disease. Prospective study and meta-analysis. *European Heart Journal*; **25**: 252-259.
- Madan, R., Gupta, B., Saluja, S., Kansr, U.C., Tripathi, B.K., Guliani, B.P. (2010). Coagulation Profile in Diabetes and its

- Association with Diabetic Microvascular Complications. *Journal of the Association of Physicians of India*; **58**: 481-489.
- Madiraju, Anila, K., Erion, Derek, M., Rahimi, Yasmeen, Zhang, Xian-Man, Braddock, Demetrios, T., Albright, Ronald, A., Prigarro, Brett, J., Wood, John, L., Bhanot, S., MacDonald, Michael, J., Jurczak, Michael, J., Camporez, Joao-Paulo, Lee, Hui-Young, Cline, Gary, W., Samuel, Varman, T., Kibbey, Richard, G., Shulman, Gerald, I. (2014). "Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase". *Nature*; **510 (7506)**: 542-546.
- Martines, F.M., Visseren, F.L., Lemay, J., *et al.* (2002). Metabolic and additional vascular effects of thiazolidinediones. *Drugs*; **62 (10)**: 1463-1480.
- Meltzer, M.E., Doggen, C.J., de Groot, P.G., Rosendaal, F.R., Lisman, T. (2009). Reduced plasma fibrinolytic capacity as a potential risk factor for a first myocardial infarction in young men. *British Journal of Haematology*; **145 (1)**: 121-127.
- Misbin, R.I., Green, L., Stadel, B.V., Gueriguian, J.L., Gubbi, A., Fleming, G.A. (1998). Lactic acidosis in patients with diabetes treated with metformin. *New England Journal of Medicine*; **338**: 265-266.
- Morrish, N.J., Wang, S.L., Stevens, L.K., Fuller, J.H., Keen, H. (2001). Mortality and Causes of Death in the WHO Multinational Study of Vascular Disease in Diabetese. *Diabetologia*; **44 (2)**: S14-S21.
- Mwambungu, A., Kaile, T., Koroloval, L., Kwenda, J., Marimo, C. (2013). A screening test for hypercoagulability in type 2 Diabetes Mellitus patients. *Asian Multidisciplinary Academic Research Journal*; **1 (14)**: 101-103.
- Nigel, Key., Michael, Makris., *et al.* (2009). Practical Hemostasis and Thrombosis. Wiley-Blackwell: 2.
- Osende, J.I., Badimon, J.J., Fuster, V., Herson, P., Rabito, P., Vidhun, R., *et al.* (2001). Blood thrombogenicity in type2 diabetes mellitus patients is associated with glycemic control. *Journal of American College of Cardiology*; **38 (5)**: 1307-1312.
- Pallister, C.J. and Watson, M.S. (2010). Haematology. Scion Publishing: 334-347.
- Radke, P.W., Schunkert, H. (2010). Diabetics with acute coronary syndrome: advances, challenges, and uncertainties. *European Heart Journal*; **31**: 2971-2973.
- Reusch, J.E. (2002). Current concepts in insulin resistance, type 2 diabetes mellitus, and the metabolic syndrome. *American Journal of Cardiology*; **90**: 19G - 26G.
- Roffi, M., Angiolillo, D.J., Kappetein, A.P. (2011). Current concepts on coronary revascularization in diabetic patients. *European Heart Journal*; **32**: 2748-2757.
- Roglic, G., Unwin, N., Bennett, P.H., Mathers, C., Tuomilehto, J., Nag, S. (2005). The Burden of Mortality Attributable to Diabetes. *Diabetes Care*; **28 (9)**: 2130-2135.
- Sahli, D., Eriksson, J.W., Boman, K., Svensson, M.K. (2009). Tissue plasminogen activator (t-PA) activity is a novel and early marker of asymptomatic LEAD in type 2 diabetes. *Thrombosis Research*; **123**: 701-706.
- Sambola, A., Osende, J., Hathcock, J., Degen, M., Nemerson, Y., Fuster, V., *et al* (2003). Role of risk factors in the modulation of tissue factor activity and blood thrombogenicity. *Circulation*; **107**: 973 - 977.
- Sapkota, B., Shrestha, S.K., Poudel, S. (2013). Association of activated partial thromboplastin time and fibrinogen level in patients with type II diabetes mellitus. *BMC Research Notes*; **6 (485)**: 1-5.
- Seljeflot, I., Larsen, J.R., Dahl-Jorgensen, K., Hanssen, K.F., Arnesen, H. (2006). Fibrinolytic activity is highly influenced by long-term glycemic control in type 1 diabetic patients. *Journal of Thrombosis and Haemostasis*; **4**: 686-688.
- Standeven, K.F., Ariens, R.A., Whitaker, P., Ashcroft, A.E., Weisel, J.W., Grant, P.J. (2002). The effect of dimethylbiguanide on thrombin activity, FXII activation,



- fibrin polymerization, and fibrin clot formation. *Diabetes*; **51**: 189-197.
- Stengenga, M.E., van der Crabben, S.N., Dessing, M.C., Pater, J.M., van der Pangaart., P.S., de Vos, A.F., *et al.* (2008). Effect of acute hyperglycaemia and/or hyperinsulinaemia on proinflammatory gene expression, cytokine production and neutrophil function in humans. *Diabetes Medicine*; **25**: 157-164.
- Stratmann, B and Tschoeoe, D. (2009). Atherogenesis and Atherothrombosis—focus on diabetes mellitus. *Best Practice and Research in Clinical Endocrine Metabolism*; **23**: 291-303.
- Stumvoll, M., Nurjhan, N., Perriello, G., *et al.* (1995). Metabolic effects of metformin in non-insulin-dependent diabetes mellitus. *New England Journal of Medicine*; **333** (9): 550 – 554.
- Tessari, P., Kiwanuka, E., Million, R., Vettore, M., Puricelli, L., Zanetti, M., *et al.* (2006). Albumin and fibrinogen synthesis and insulin effect in type 2 diabetic patients with normoalbuminuria. *Diabetes Care*; **29**: 323-328.
- Thogersen, A.M., Jansson, J.H., Boman, K., Nilsson, T.K., Weinehall, L., Huhtasaari, F., *et al.* (1998). High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor. *Circulation*; **98** (21): 2241-2247.
- Tripathy, B.B., Chandalia, H.B., Das, A.K., Rao, P.V., Mohan, V. (2012). RSSDI Textbook of Diabetes Mellitus 2nd ed. Japee Brothers Medical Publisher New Dehi:235.
- Vazzana, N., Ranalli, P., Cucurullo, C., Davi, G. (2012). Diabetes mellitus and thrombosis. *Thrombosis Research*; **129**: 371–377.
- Verkleij, C.J.N., Gerdes, V.E.A., de Bruijn, R., Meijers, J.C.M., Marx, P.F. (2009). The hemostatic system in patients with type 2 diabetes with and without cardiovascular disease. *Journal of Thrombosis and Haemostasis*; **7** (2): PP-WE-336.
- Wolberg AS, Campbell RA. Thrombin generation, fibrin clot formation and hemostasis. *Transfusion Apheresis Science*; **38**: 15-23.
- Yamagashi, S.M., Ueda, S., Makamura, K., Imaizumi, T. (2007). Advanced glycation end products and cardiovascular disease in diabetes. *Cardiovascular and Hematological Agents Medicinal Chemistry*; **5**: 236-240.
- Yokoyama, H., Myrup, B., Rossing, P., Ostergaard, P.B. (1996). Increased tissue factor plasminogen activity in insulin dependent diabetes mellitus patients with nephropathy. *Diabetes Care*; **19**: 441-443.

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